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FURTHER OBSERVATIONS ON THE DISPOSAL OF EXCRETA (THIRD PAPER).[a]

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Testing the viability of hookworm eggs is quite different in certain respects from testing the viability of typhoid bacilli. In the latter case cultural methods may be utilized to bring into evidence (by multiplication) the germs that remain after a given time; in the case of hookworms, however, the observer is dependent upon microscopic examination (either judgment based upon the appearance of the eggs under the microscope, or the finding, by microscopic examination, of embryos or larvæ which have hatched out, but not multiplied, in cultures). Thus, as time goes on, and as the eggs and larvæ gradually die and become unrecognizable, there remain fewer and fewer objects upon which to base conclusions. The work therefore becomes increasingly more tedious and more difficult from week to week, and in case of negative findings it is, from the nature of things, not absolutely reliable.

The literature on the viability of the typhoid bacillus is so lacking in uniformity as respects results that one may prophesy a similar, or possibly even greater, difference in conclusions as authors study the viability of various zooparasitic infections.

The results presented in the present paper were obtained chiefly at unheated room temperature at the United States Marine Hospital station at Wilmington, N. C., during the summer and early fall of 1910. An accident to some of our cultures prevents us from giving a complete continuation of the observations published in our two earlier papers, but the results permit certain conclusions of a practical nature.

It has been our purpose to determine if possible about what may be expected in respect to the viability of *Necator americanus* under actual conditions as they exist in eastern North Carolina, and on this account no attempt has been made to keep the cultures at a uniform, artificial temperature, or under theoretically ideal conditions. On the contrary, certain experiments have purposely been conducted under irregular conditions, simulating those found in nature, as, for instance, in a cotton-mill village.

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[a] For previous papers see Public Health Reports, Vol. XXV, No. 27, July 8, 1910, pp. 947-950, and No. 33, August 19, 1910, pp. 1137-1140.

EFFECTS OF DRYING.

It has been an established fact for some time that the eggs and encysted larvæ of hookworms (*Uncinariinæ*) in general are killed by becoming completely dried. Experiments to this effect have been conducted, purposely or accidentally, in several laboratories. The question has arisen in our minds: Given the ordinary surface privy, such as is so general in this locality, how long will the encysted hookworm larvæ live in the sand, subject, as the sand is, to varying conditions? Several of our experiments bear upon this point, but do not as yet settle it definitely.

Culture 8.—A sample of sand from under a surface privy was placed (June 20) in an 8-ounce bottle. It contained live hookworm embryos. It was kept at room temperature and had partially dried on July 3, when water was added. On July 19, motionless larvæ were present; some of them were dead; none was positively recognized as alive. It was allowed to dry out gradually until November 15, when water was added and the culture placed in the incubator. On November 18, 10 slides showed 2 well-preserved, but motionless, encysted larvæ. It can not be asserted whether these were alive or dead. It is, however, striking that apparently perfectly preserved encysted larvæ were found in this sand 151 days after it was collected, although the culture had been (apparently) completely dried out once and partially dried out once. Had these larvæ been dead long, they would not have been so well preserved; in fact, only their skin might have remained. The observation raises the suspicion that the encysted larvæ may possibly live about as long as five months in the privy sand, even when some of the conditions (as occasional drying) are unfavorable. This statement, of course, does not mean that the encysted stage will withstand drying for five months.

Culture 15.—Flyblown fecal material was placed in a bucket and covered with 6½ inches of sterilized sand. As reported in an earlier communication, 15 flies (*Ophyra leucostoma*) issued and came through the sand and (July 16) live encysted larvæ were found on the surface. The sand was well moistened and allowed to stand at unheated room temperature until November 12, when it was dry; water was then added. Numerous microscopic examinations (November 13, 14, 16) at different depths failed to show any signs of hookworms.

Thus from July 16 to November 13 (120 days) all trace of hookworm infection was lost. As stated in the introductory paragraph, however, negative findings in this line of work are not to be relied upon implicitly.

Culture 9.—A large sample of fecal material containing eggs of *Ascaris lumbricoides* and *Necator americanus* was placed (June 20) in a jar with sterilized sand; encysted hookworm larvæ were observed June 26. From July 20 to November 14 no water was added to No. 9, in which active hookworm larvæ were plentiful on July 3.

On November 14 No. 9 was entirely dry, and water was added. Microscopic examination on November 18 showed undetermined live nematodes (not *Necator* or *Ascaris*), but no trace of hookworms was found.

Subculture 9a.—On June 26 a sample of No. 9 was transferred to fresh sterilized sand. July 3 many active hookworm larvæ were present; July 16 *Ascaris* embryos were observed in the egg and hookworm larvæ were alive. On November 12 and 15 *Ascaris* embryos (alive?) were found, but there was no trace of hookworms. Thus, after 145 (148) days from the time of the original culture (June 20) hookworm infection could not be traced. It is not positively excluded that this culture became somewhat dry at one time in September.

Subculture 9b.—July 3 a sample of No. 9 was transferred to fresh sterilized sand. On July 16 live hookworm larvæ were present. The culture was kept at unheated room temperature until November 12, when it was incubated. On November 12, 15, and 17 microscopic examination failed to show live hookworm infection, but dead hookworm larvæ were found; live *Ascaris* embryos were present in the eggs on November 17. It is not positively excluded that this culture became rather dry at one time in September. Thus, 150 days (November 17) after the original culture (No. 9, June 20) the active *Ascaris* infection had not died out, but no trace of live hookworm infection remained, although dead hookworm skins were visible.

Culture 4.—Fecal material containing eggs of *Ascaris* and of *Necator* was placed (June 18) in a bucket of water, to which a film of kerosene was added. As stated in an earlier communication, live hookworm eggs were present 33 days later.

Subculture 4b.—A sample of No. 4 was transferred (June 23) to sterilized sand, in which live hookworm larvæ were observed July 3 and 16. The culture was kept at unheated room temperature until November 12, and was placed in the incubator November 16. It is possible that this culture became rather dry in September. Repeated examinations (November 12 to 18) failed to reveal any live hookworm infection, although a few dead larval skins were found November 18; one *Ascaris* egg contained a live motile embryo November 17.

Thus (4*b*) in moist, sterilized sand (which may possibly have become rather dry on one occasion) kept at unheated room temperature from June 23 to November 12 (18), namely, 142 (148) days, live *Ascaris* infection was present, but no live hookworm infection could be found.

Subculture 4f.—On July 20, of 12 hookworm eggs taken from No. 4, 11 were considered dead, 1 doubtful. A portion of No. 4 was transferred (July 20) to sterilized sand (4*f*), which was kept at unheated room temperature until November 12, then incubated. It is possible that this became rather dry once in September. Repeated examinations November 12, 15, and 19 revealed only one hookworm egg (November 15) which was dead.

Thus 117 days after 4*f* was prepared a hookworm egg was recognized, but no live infection was found.

Culture 5.—Fecal material containing live *Ascaris* eggs and live *Necator* eggs was placed (June 18) in a jar of water, to which a film of kerosene was added. Live and undeveloped eggs of *Ascaris* and *Necator* were observed on July 16; some of the hookworm eggs were dead on that date. The jar was allowed to dry out. On November 12 it was completely dry. On November 14, 20, and 21, 15 preparations showed numerous *Ascaris* eggs, some of which contained embryos; most of the eggs were dead, but in several instances this point could not be decided; in at least three instances (November 21) the eggs appeared to be perfectly normal and alive. Thus the *Ascaris* infection had not entirely died out in 156 days, although the material had been quite dry.

No sign of hookworm infection could be distinguished.

EFFECTS OF DECOMPOSITION.

Personally we incline more and more to the utilization of decomposition and fermentation as a method of destroying hookworm infection in fecal material, and on this account we are following the eggs kept in water for various periods of time. Fecal material is collected from privies at various intervals, placed in buckets of water, and allowed to stand at unheated room temperature. In former communications it has been shown that dead eggs may be found after 4 days, but that live eggs may be found after 33 days. We have now found live hookworm eggs as late as 70 days.

Culture 33.—Fecal material was placed (September 7) in a bucket of water and allowed to stand at unheated room temperature until November 12. Microscopic examinations on November 12, 13, 16, and 17 showed a large number of dead hookworm eggs, but 2 were found which were probably alive and 2 were found (November 16) which were undoubtedly alive. A *Hymenolepis nana* egg was probably dead.

Subculture 33a.—A portion of No. 33 was transferred (November 13) to sterilized sand and incubated until November 21. Examinations made November 20 and 21 showed 31 hookworm eggs, of which 30 were surely dead, 1 probably dead. A *Hymenolepis nana* egg may have been dead, but this is uncertain. One *Ascaris* egg was dead; 2 were doubtful.

From these observations it must be concluded that while the mass of hookworm eggs will die when the feces are subjected for 70 days to decomposition in water, some few may still be found alive.

Culture 32.—Fecal material was placed (August 13) in a bucket of water and allowed to stand at unheated room temperature until November 12. On November 13, 20 slides showed 4 dead hookworm eggs. Thus, so far as the examination determined, the hookworm infection had died out in 68 days. In view of the observations recorded for No. 33 the results obtained in No. 32 must be considered as of secondary value.

Culture 27.—Fecal material containing eggs of *Ascaris* and of *Necator* was placed (July 20) in a bucket of water to which a film of kerosene was added (July 21). No water was added later. This culture was kept at unheated room temperature until November 12. The water had evaporated until the material was soft-mushy in character. In 13 preparations (November 14) 20 hookworm eggs and 34 *Ascaris* eggs were found; all of the former and 29 of the latter were dead; 5 *Ascaris* eggs may possibly have been alive.

Subculture 27a.—A portion of No. 27 was transferred (November 14) to sterilized sand and incubated. Six preparations (November 18) showed 27 hookworm eggs, all of which were dead, and 27 *Ascaris* eggs, 20 of which were dead, 7 probably dead.

Thus, after 117 (121) days all of the hookworm infection and at least 80 per cent of the *Ascaris* infection appear to have died.

Culture 17.—Fecal material containing eggs of *Ascaris* and *Necator* was placed (June 22) in a bucket of water and allowed to stand in the open until November 12, when 15 preparations showed 25 hookworm eggs and 5 *Ascaris* eggs, all of which were dead. One of the hookworm eggs contained a well-developed embryo, but this is the only instance we have found in any of our observations in which a hookworm egg developed so far in water.

Subculture 17a.—A portion of No. 17 was transferred (November 13) to sterilized sand and incubated. Microscopic examination (November 18) showed no embryonic development, but in 5 preparations 5 dead hookworm eggs were found.

Thus in 144 (149) days after the original culture all hookworm eggs found were dead.

CHLORIDE OF LIME.

To test the effect of chloride of lime upon hookworm eggs fecal material was mixed in water in buckets, each containing about 10½ quarts (about 10,000 cc.), and 1, 5, and 10 even tablespoonsful of chloride of lime were added (Nos. 39, 41, 42).

Experiment 42.—After 22 hours, the egg in bucket 42, with 10 tablespoonsful of chloride of lime, appeared perfectly normal. Some of the fluid was placed (4 p. m., November 18) in the incubator, and when examined (11 a. m., November 20) contained free, live embryos. Live embryos were also present at noon, November 21.

Experiment 39.—On account of the appearance of the eggs in No. 42 after 22 hours there was added, at 6 p. m., November 18, to No. 39 (which had only one tablespoonful of chloride of lime) half a can (approximately quarter of pound) of the disinfectant. At 5 p. m. November 19, at 10 a. m. November 20, and at noon November 22 the eggs appeared perfectly normal.

Subexperiment 39a.—At 5 p. m. November 19 (after 23 hours action of the strengthened disinfectant) some of the fluid was placed in the incubator, and at 10 a. m. November 20, live free embryos were present. At noon November 21, 5 embryos observed were dead.

Subexperiment 39b.—At 10 a. m. November 20 (after 40 hours action of the strengthened disinfectant) some of the fluid of No. 39 was placed in the incubator, and when examined at 4 p. m. November 22, free live embryos were found.

Subexperiment 39c.—At noon November 22 (after 90 hours of action of the strengthened disinfectant) some of the fluid from No. 39 was placed in the incubator; the hookworm eggs appeared to be normal. Report on incubation will appear later.

FLIES.

In a former communication we reported that if fly-blown fecal material is buried under 6½ inches of sterilized sand, flies (*Ophyra leucostoma*) will crawl to the surface and issue.

Experiment 21.—Fly-blown fecal material was buried (July 20) in a large screened glass jar under 17 inches of sterilized sand. On August 4 three adult flies issued at the surface. These were determined by Mr. Coquillett as *Sarcophaga* sp.

The two following experiments are reported with a certain amount of academic reserve, as the supply of sterilized sand was exhausted at the moment that the material was available. The experiments are being repeated.

Experiment 29.—Fly-blown fecal material was buried (July 21) in a screened standpipe under 48 inches of clean (unsterilized) sand.

Within less than 24 hours some insects came to the surface, but they were not disturbed. Within 10 days adult flies began to issue at the surface, and continued to appear until 37 were collected (August 4). They have been determined by Mr. Coquillett as the common house fly (*Musca domestica*).

Experiment 30.—Fly-blown fecal material was buried (August 3) in a screened standpipe under 72 inches of clean (unsterilized) sand. On August 24 an adult fly appeared at the surface, and flies continued to issue until 4 appeared. They were left in the jar awaiting the issuance of other insects, and when collected finally were unfortunately too broken to permit proper identification beyond the fact that they were flies.

SUMMARY.

The foregoing observations indicate that—

(1) It is not safe, at present, to assume that the sand under and around a privy is entirely free from hookworm infection about 5 months (151 days) after the privy was last used.

(2) After about 4 months (120 days), however, the infection may be very greatly reduced, and possibly in some instances entirely dead.

(3) After about 5 months (150 days) in sand, live *Ascaris* embryos in the eggs may be found, even when all the hookworm larvæ observed are dead. *Ascaris* eggs, apparently alive and normal, were found after 156 days.

(4) Hookworm eggs may be identified in sand cultures 117 days old.

(5) When fecal material is subjected to decomposition in water for 70 days (namely, about 2⅓ months), the mass of hookworm eggs die, but a few can survive.

(6) No hookworm egg has as yet been found alive in feces subjected to decomposition for 117 to 149 days, namely, about 4 to 5 months.

(7) It seems very probable, from present data, that under conditions under which our observations have been conducted, if fecal material containing hookworm eggs is subjected to decomposition in water for about 3 months, all hookworm infection will be dead. If this probability is confirmed by further observations, then viewed from the standpoint of hookworm infection alone, the effluent from the L. R. S. sanitary privy described in Public Health Reports, 1910, pages 1619–1623, should be stored 3 months before being used as fertilizer.

(8) In feces allowed to decompose in water, *Ascaris* eggs resist the decomposition better than do the hookworm ova. At the end of about 4 months (117 to 121 days), however, at least 80 per cent of the *Ascaris* eggs appear to be dead.

(9) The action of chloride of lime in the strength of approximately one-fourth pound to 10½ quarts of water for 22 to 40 hours does not kill hookworm eggs.

(10) If fly-blown fecal material is buried under 17 inches of sterilized sand, flies (*Ophrya leucostoma*) will crawl through the sand and complete their development.

(11) When fly-blown fecal material was buried under 48 inches of clean (unsterilized) sand, flies (*Musca domestica*) issued from the surface.

(12) When fly-blown fecal material was buried under 72 inches of clean (unsterilized) sand, flies (genus and species undetermined) issued from the surface.